June 19, 2018

Kristina Thayer, Ph.D.
Director, Integrated Risk Information System (IRIS)
National Center for Environmental Assessment
USEPA Headquarters
Ariel Rios Building 1200 Pennsylvania Avenue, N.W.
Mail Code: 8601P
Washington, DC 20460

By email (thayer.kris@EPA.gov) and submission to EPA Docket Nos. EPA-HQ-ORD-2014-0313 and EPA-HQ-ORD-2010-0540

RE: Draft IRIS Assessment of Hexavalent Chromium (Chromium VI)
Docket ID Nos. EPA-HQ-ORD-2014-0313 and EPA-HQ-ORD-2010-0540

Dear Dr. Thayer,

Following up on my correspondence from March 21, 2018 and April 30, 2018, the American Chemistry Council's (ACC) Hexavalent Chromium Panel (Panel) has concerns about how the IRIS program will incorporate the large toxicogenomics dataset sponsored by the Panel into the assessment of hexavalent chromium [Cr(VI)]. The mode of action (MOA) researchers communicated with Dr. Lyle Burgoon, formerly at EPA, from 2014 – 2016 concerning this Cr(VI) toxicogenomics dataset, and in my last correspondence I requested a meeting with EPA to discuss the following concerning use of the dataset to inform the MOA for tumors observed in a 2-year rodent cancer bioassay:

1. A timeline of events relevant to the Cr(VI) genomics data;
2. An explanation for the varying number of microarrays available through different online data repositories; and
3. A general findings comparison between published analyses.

Since the IRIS program has declined my request for a meeting, we are submitting this information into the docket for the Cr(VI) IRIS assessment.

Timeline of events relevant to Cr(VI) genomics data

Table 1 summarizes the timeline of events surrounding the Cr(VI) genomics data and clarifies any potential discrepancies between data repositories over time and differences in analysis strategies used in different publications.

Table 1. Timeline summarizing events involved in the Cr(VI) genomics dataset.

DATE	EVENT	RELEVANT WEBSITE[1]
2012	Two studies were published using: 1. Mouse duodenum and jejunum data (Kopec et al. 2012a) 2. Mouse and rat duodenum and jejunum data (Kopec et al. 2012b). Data from manuscripts were deposited in dbZach, an internal data management system that is compliant with the Minimum Information About a Microarray Experiment (MIAME) standard (Burgoon et al. 2006).	dbZach

2014	Data were provided online through Cr6study.info. Data included:
	<u>1. qRT-PCR data used in the Kopec (2012a,b) publications</u>
	
	<u>2. Raw genomics microarray data used in the Kopec (2012a,b) publications</u>
	• All raw microarray data (as .gpr files) for the mice and rat, oral palate, duodenum, and jejunum (day 8 and 91), alongside a metadata file
	
	<u>3. Genomics analysis code used in the Kopec (2012a,b) publications</u>
	• Note that this analysis included data from both array dyes (Cy3 and Cy5) and data were processed using in-house code and algorithms, which are provided in Cr6study.info, and include:
	  ○ SAS code used to analyze microarray data (note: input files with appropriate tags referred to in the code were not originally provided – see 2018 update)
	  ○ R code to calculate P1(t) statistics
	
	<u>4. Genomics analysis statistical results used in the Kopec (2012a,b) publications</u>
	• Excel worksheets containing all array probesets and their corresponding fold change values and P1(t) statistical results for the rat and mouse, duodenum and jejunum, at day 8 and 91
		Cr6study.info

APRIL 2016	A labeling error was identified in the raw genomics data for the rat within the files in Cr6study.info.	Cr6study.info

DATE	EVENT	RELEVANT WEBSITE[1]
	• Specifically, incorrect probeset identifiers were included in the rat raw array data files (.gpr files). These identifiers were corrected, and Dr. Burgoon was notified of this edit via email on January 8, 2016 and April 5, 2016 (see ATTACHMENT A) • This labeling error had no effect on the analyses or interpretation reported in Kopec et al. (2012b)	
AUGUST 2016	ToxStrategies received a request from Stiven Foster at the US EPA to access data from the Cr6study.info website. Access was granted on August 10, 2016 (see ATTACHMENT A).	Cr6study.info
SEPTEMBER 2016	All raw and processed array files were uploaded into the NCBI's Gene Expression Omnibus (GEO) repository. These data included: • All raw microarray files • Processed array data, consisting of quantile normalized expression data derived from the Cy3 array signal. Note: these processed array data were updated in 2016 and differ from those used in the Kopec (2012a,b) publications. Updated results from these data were published (Thompson et al. 2016, Rager et al. 2017, described below) Data were uploaded alongside a reference to the Thompson et al. (2016) publication (final publication on November 2016, below)	GEO
NOVEMBER 2016	A study was published using the mouse and rat palate data (Thompson et al. 2016), using the updated array processing strategy provided in GEO.	GEO
MAY 2017	A study was published using the mouse duodenum data (Rager et al. 2017), using the updated array processing strategy provided in GEO.	GEO
FEBRUARY 2018	The GEO posting was updated to include additional citation information, referencing Rager et al. (2017).	GEO
FEBRUARY 2018	The link to the raw microarray files was temporarily not working through Cr6study.info. This link was corrected.	Cr6study.info
FEBRUARY 2018	More data were provided to further support the analysis conducted in the Kopec (2012a,b) publications. • To detail, the original SAS code used in the genomics statistical analysis was originally provided in the 2014 data upload. This update added the specific input files that were used to execute the SAS code.	Cr6study.info

[1] website URLs are Cr6study.info (https://cr6study.info), dbZach (http://dbzach.fst.msu.edu/), GEO (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE87262).

Explanation for varying numbers of arrays on different websites

The number of array samples included at Cr6study.info, representing the analysis published in the Kopec et al. (2012a,b) publications, differs slightly from the number of array samples included in the GEO repository, representing the analyses published in the recent publications (Rager et al. 2017, Thompson et al. 2016). Specifically, the arrays in **Table 2** were excluded from the GEO repository.

Table 2. Arrays that are included in Cr6study.info but excluded in the GEO repositories.

GPR file	Species, Tissue, and Duration Group	Array Components[1]	Note
4417.gpr	Mouse duodenum day 91	Cy3 signal: animal ID 3426, control Cy5 signal: animal ID 3401, exposed at 4 mg/L dose	Duplicate
4418.gpr	Mouse duodenum day 91	Cy3 signal: animal ID 3401, exposed at 4 mg/L dose Cy5 signal: animal ID 3426, control	Duplicate
4451.gpr	Mouse duodenum day 91	Cy3 signal: animal ID 3416, exposed at 14 mg/L dose Cy5 signal: animal ID 3426, control	Technical replicate
4452.gpr	Mouse duodenum day 91	Cy3 signal: animal ID 3421, exposed at 60 mg/L Cy5 signal: animal ID 3426, control	Technical replicate
4453.gpr	Mouse duodenum day 91	Cy3 signal: animal ID 3409, exposed at 170 mg/L Cy5 signal: animal ID 3425, control	Technical replicate
4454.gpr	Mouse duodenum day 91	Cy3 signal: animal ID 3400, control Cy5 signal: animal ID 3404, exposed at 520 mg/L	Technical replicate

[1] Note that data from the Cy3 and Cy5 dye signals were used in the Kopec et al. (2012a,b) publications; while analyses only focused on data from the Cy3 dye signal in the Rager et al (2017) and Thompson et al. (2016) publications to minimize potential dye bias / interference issues.

These arrays in Table 2 were excluded from the GEO repository, reflective of the more recent Rager et al. (2017) publication, because these arrays represented duplicated data or technical replicates. Specifically, chip scan data in array IDs 4417 and 4418 were identified as duplicated data; thus both of these arrays were removed from the analysis. Array IDs 4451, 4452, 4453, and 4454 represented technical replicate arrays used for internal quality purposes. These arrays measured gene expression profiles of samples from animals that were already accounted for by

other arrays. Neither the Kopec et al. nor the Rager et al. analyses included technical replicate arrays.

The biological replicates were explained in the Rager et al. (2017) publication in the methods section:

> *"RNA samples were assessed in biological triplicate in all dose groups (0, 0.03, 4, 14, 60, 170, and 520 mg/l SDD at day 8 and day 91) except for the 4 mg/l SDD exposure group from day 91, which was analyzed in biological duplicate due to potential microarray quality issues."*

The arrays that were removed due to duplicate issues or represented technical replicates were excluded from the data in the GEO repository, to be consistent with the Rager et al. (2017) publication. The number of biological replicates used in this analysis is detailed in Table 3. All array data, including duplicate data/technical replicates, are included in Cr6study.info.

Table 3. Biological replicate numbers in the GEO database, reflective of the array analysis in Rager et al. (2017).

Duration Group	Species, Tissue	Dose (mg/L sodium dichromate dihydrate)							
		0	0.3	4	14	60	170	520	Total
8 days	Mouse, duodenum	3	3	3	3	3	3	3	21
91 days	Mouse, duodenum	3	3	2[1]	3	3	3	3	20

[1] Note that the analyses conducted by Kopec et al. (2012a,b) contained one more biological replicate in this dose group.

General comparison between publication findings

Genomics data analysis methods and standards have evolved over time. Thus, the data processing and statistical methods differed between the original publications using the Cr(VI) genomics data in the mouse duodenum (Kopec et al. 2012a,b) and more recent, updated publication (Rager et al. 2017); however, overall general findings remained consistent despite these varying approaches. For example, Kopec et al. 2012a, which focused on the mouse intestinal responses, identified genes involved in oxidative stress and cytotoxicity with altered expression, representing early key events. Other pathways noted as enriched included those involved in cell cycle, lipid metabolism, and immune response. Pathways involved in DNA repair were also noted in the day 8 results, including the nucleotide excision repair pathway, which did not show enrichment at day 91 (Kopec et al. 2012a). Similarly, Kopec et al. (2012b) noted pathways relevant to oxidative stress, immune response, protein synthesis, cell cycle/cell growth and proliferation, and DNA damage and repair in the mouse and rat duodenum and jejunum.

Similar results were apparent in the Rager et al. (2017) analysis focusing on the mouse duodenum, with pathways involved in cell stress and injury, cell death, and cell growth, proliferation and

development involved in early key events that were also sustained in the day 91 results. The nucleotide excision repair pathway was also identified as enriched in the day 8 results, but not the day 91 results; and it was noted that this pathway included eight genes showing increased expression associated with Cr(VI), all of which were involved in general DNA transcription and cell cycle signaling. Pathways involved in metabolism and immune response were also identified as enriched, similar to Kopec et al. (2012a). **In summary, analysis strategies have evolved over time surrounding the use of the Cr(VI) genomics data; still, general findings have remained consistent despite varying approaches.**

In conclusion, the raw and processed Cr(VI) genomics data are provided online, and the methods used to analyze these data have evolved over time to parallel ongoing advances in genomics-based assessments. This robust data set provides important information that can be used to further understand the MOA of Cr(VI). We have been fully transparent about the research conducted, including making the raw genomics data as well as other raw data available to EPA and other scientists.

If you have any questions, please contact me at eileen_conneely@americanchemistry.com or at 202-249-6711.

Sincerely,

Eileen Conneely

Eileen Conneely, M.P.H., J.D.
Director, Chemical Products & Technology Division
American Chemistry Council

Attachment A: Email communication on updates to Cr6study.info genomics data

cc: C. Gibbons, gibbons.catherine@epa.gov
A. Sasso, sasso.alan@epa.gov
R. Yamada, yamada.richard@epa.gov
J. Orme-Zavaleta, orme-zavaleta.jennifer@epa.gov
T. Bahadori, bahadori.tina@epa.gov
E. Ohanian, ohanian.edward@epa.gov

ATTACHMENT A

Email communication on updates to Cr6study.info genomics data

Subject: Cr(VI) raw microarray data
Date: Thursday, March 13, 2014 at 12:37:11 PM Central Daylight Time
From: Apple-Mail=_405249D0-D0F5-4909-96A9-35F59E23F6BE Mark Harris boundary=
To: Burgoon.Lyle@epa.gov
CC: Rusty Thomas, Tim Zacharewski, Gibbons.Catherine@epa.gov, Chad Thompson, Deb Proctor

Lyle: as I mentioned in the meeting on Monday, the data will be posted to our new Cr(VI) MOA website before SOT. Once the site is live I will send you all a link to it. You will be able to download the genomics data at that time.

The approach and methods that were used to analyze the data are adequately described in the Kopec papers. Our use of the genomics data to support the Mode of Action can be found in our Critical Reviews in Toxicology paper. Links to these Open Access papers can be found at the web page below:

http://www.toxstrategies.com/publications/CRVI_MOA_study.htm

mark

From: "Burgoon, Lyle" <Burgoon.Lyle@epa.gov>
Date: March 12, 2014 at 8:43:16 AM CDT
To: "cthompson@toxstrategies.com" <cthompson@toxstrategies.com>
Cc: "tzachare@msu.edu" <tzachare@msu.edu>, "Gibbons, Catherine" <Gibbons.Catherine@epa.gov>
Subject: Cr(VI) raw microarray data

Dr. Thompson,

It was nice seeing you and Dr. Harris (via video) at Monday's meeting @ NCEA HQ. I was quite pleased to hear Ms. Mason state that ACC expected the researchers would share their data and results with NCEA.

I'm following up on our discussion from Monday and am requesting access to all of your raw microarray data, as well as your analyzed data that supports the conclusions in your papers. In addition, it would be helpful if you could also supply us with the analysis code that was used, any protocols used for the analyses, and any other supporting documentation that may help us understand how the assays and analyses were performed.

For clarity, I am using the MIAME definition of "raw data", and my request for the additional information is in line and keeping with the MIAME standard, which can be found here: http://www.mged.org/Workgroups/MIAME/miame.html.

To facilitate data transfer, I can set-up an EPA-based FTP site where you can upload the data.

Thanks again for presenting your latest results to us, and I look forward to receiving the data.

Cheers,

Lyle

Lyle D. Burgoon, Ph.D
National Center for Environmental Assessment
Chief, Hazardous Pollutant Assessment Group (Acting)
US Environmental Protection Agency
Phone: 919.541.7808
Fax: 919.685.3473
Cell: 919.397.8036

--

Note New Address
ToxStrategies, Inc.
Innovative Solutions > Sound Science

Mark Harris, Ph.D.
Principal Health Scientist

23123 Cinco Ranch Blvd, Suite 220
Katy, TX 77494

Direct Dial: (281) 394-1567
Houston Office: (281) 712-2062 Ext 2001
Cell: (832) 868-7729
fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

Subject: Cr(VI) MOA Study Website
Date: Monday, March 24, 2014 at 8:34:03 AM Central Daylight Time
From: Apple-Mail=_B33E4A44-0724-416B-A519-C1B4A7F9AE29 Mark Harris boundary=
To: Burgoon.Lyle@epa.gov
CC: Tim Zacharewski, Gibbons.Catherine@epa.gov, Chad Thompson, Rusty Thomas

Lyle: our Cr(VI) MOA study website is live. You can access is at www.cr6study.info

Under the Study Data tab you can register to download data. Once registered, you will receive a password to access the data download portion of the site.

Not all of the genomics data you requested has been posted. Things that are not posted yet are:

1. Analysis Code
2. QRT-PCR data

These items will be added in the next few weeks.

mark

~~

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Cell: (832) 868-7729
fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

Subject: Re: Cr(VI) MOA Study Website
Date: Thursday, April 3, 2014 at 3:07:45 PM Central Daylight Time
From: Mark Harris
To: Burgoon, Lyle
CC: Gibbons, Catherine, Vandenberg, John, Flowers, Lynn

I will have to talk to our legal folks about this.

Back in touch shortly.

mark

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fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

On Apr 3, 2014, at 2:59 PM, Burgoon, Lyle <Burgoon.Lyle@epa.gov> wrote:

> Hi Mark,
>
> We cannot access the data at this time due to our inability to agree to the Terms and Conditions associated with data access found here: http://cr6study.info/study-data/terms-and-conditions/
>
> Specifically, we request that ToxStrategies delete the indemnification clause in the Terms and Conditions. As written, the Agency cannot agree to the terms as it would be a violation of the Anti-Deficiency Act (i.e., making a commitment to an unknown future cost is a direct violation of the Anti-Deficiency Act).
>
> Please let me know what action you are willing to take as soon as possible. As you are aware,

time is of the essence with respect to the Cr(VI) IRIS Assessment. Your prompt attention to this matter is appreciated.

Thanks,

Lyle

Lyle D. Burgoon, Ph.D
Chief, Hazardous Pollutant Assessment Group (Acting)
National Center for Environmental Assessment
US Environmental Protection Agency
Phone: 919.541.7808
Fax: 919.685.3473
Cell: 919.397.8036

From: Mark Harris [mailto:mharris@toxstrategies.com]
Sent: Monday, March 24, 2014 9:34 AM
To: Burgoon, Lyle
Cc: Tim Zacharewski; Gibbons, Catherine; Chad Thompson; Thomas, Russell
Subject: Cr(VI) MOA Study Website

Lyle: our Cr(VI) MOA study website is live. You can access is at www.cr6study.info

Under the Study Data tab you can register to download data. Once registered, you will receive a password to access the data download portion of the site.

Not all of the genomics data you requested has been posted. Things that are not posted yet are:

1. Analysis Code
2. QRT-PCR data

These items will be added in the next few weeks.

mark

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Cell: (832) 868-7729
fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

Subject: Terms and Conditions Modified
Date: Monday, April 7, 2014 at 12:23:08 PM Central Daylight Time
From: Apple-Mail=_54682036-6E73-4A57-B537-DA70B702C164 Mark Harris boundary=
To: Burgoon, Lyle

We modified the terms and conditions for the US Government for our website http://cr6study.info. The additional text is: If you are a United States Government entity, the indemnification provision of the Terms and Conditions is hereby waived. Your liability for any breach of the Terms and Conditions, or any claim arising from the Terms and Conditions, shall be determined under the Federal Tort Claims Act, or other governing authority.

mark

--

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Cell: (832) 868-7729
fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

Subject: Website
Date: Monday, April 7, 2014 at 3:43:02 PM Central Daylight Time
From: Apple-Mail=_5CAFA6EE-E253-474E-8737-E357CC0E69C7 Mark Harris boundary=
To: Burgoon, Lyle

We have also posted the genomics analysis code to the website today. Only thing remaining is the QRT-PCR info which is still being assembled.

mark

--

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fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

Subject: T&C - how does this
Date: Wednesday, April 23, 2014 at 2:09:01 PM Central Daylight Time
From: Apple-Mail=_1704D469-54A7-4A17-9C82-0F0AADD0DE20 Mark Harris boundary=
To: Burgoon, Lyle
Attachments: Revised Cr6 Website Terms.docx

Lyle: I got some revised T&C language from the attorneys today. See attached in Tracked Changes. If this works let me know and I will get the website updated.

Also, if these words don't work, can you ask the attorney reviewing this to suggest some words that would work for the US government?

thanks

mark

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Cell: (832) 868-7729
fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

Subject: Re: T&C - how does this
Date: Tuesday, April 29, 2014 at 7:28:32 AM Central Daylight Time
From: Apple-Mail=_3480B4DA-5FF4-4C62-AE8C-422044ED03FF Mark Harris boundary=
To: Burgoon, Lyle

Lyle: the website has been updated with this revised language. Also, we have added the QRT-PCR data that we received from Tim.

mark

--

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Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

On Apr 28, 2014, at 10:08 AM, Burgoon, Lyle <Burgoon.Lyle@epa.gov> wrote:

> Hi Mark,
>
> Thanks for working with us that language meets our needs. Sorry for the delay was out sick last week.
>
> Cheers,
>
> Lyle
>
> Lyle D. Burgoon, Ph.D
> Chief, Hazardous Pollutant Assessment Group (Acting)
> National Center for Environmental Assessment
> US Environmental Protection Agency
> Phone: 919.541.7808
> Fax: 919.685.3473

Cell: 919.397.8036

Notice (If This Communication Regards a Contract): Nothing in this message shall be construed as a change to the price, schedule, or terms and conditions of the contract. If the receiver does construe it otherwise, please notify me immediately so that proper contract action can be initiated.

From: Mark Harris [mailto:mharris@toxstrategies.com]
Sent: Wednesday, April 23, 2014 3:09 PM
To: Burgoon, Lyle
Subject: T&C - how does this

Lyle: I got some revised T&C language from the attorneys today. See attached in Tracked Changes. If this works let me know and I will get the website updated.

Also, if these words don't work, can you ask the attorney reviewing this to suggest some words that would work for the US government?

thanks

mark

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Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

Subject: cr6study.info
Date: Friday, September 18, 2015 at 12:51:30 PM Central Daylight Time
From: Mark Harris
To: lyle.d.burgoon@usace.army.mil

Lyle: you should have received an approval for access to the data via email. If you have any issues downloading data just let me know. I did not realize you had changed jobs. Good luck with the new position. Why is the Army interested in Cr(VI)?

mark
--

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Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com
Linkedin: http://www.linkedin.com/in/toxstrategiesharris

Subject: Re: [EXTERNAL] cr6study.info
Date: Friday, September 18, 2015 at 3:40:14 PM Central Daylight Time
From: Mark Harris
To: Burgoon, Lyle D ERD-MS

Lyle: that is good to know about being able to collaborate on future projects. You never know when a client will ask for such analyses.

One question – is it is relatively straightforward to engage you all for a project? Meaning, if something came up, could the paperwork being done relatively quickly to get going?

mark

From: "Burgoon, Lyle D ERD-MS"
Date: Friday, September 18, 2015 at 3:35 PM
To: Mark Harris
Subject: Re: [EXTERNAL] cr6study.info

Hey Mark,

Thanks. The Army has no interest in chromium (thankfully). However, EPA is in the process of contracting with the Army to reanalyze the data. So, in preparation for that, we requested the data.

On a side note, in my new federal job I'm soft money, and we operate like a consulting company. So if in the future you need CompTox or toxicogenomic support, let me know. We can accept funds from companies for collaborative work.

If you want to chat sometime, my number is 517-303-5368.

Cheers,

Lyle

Lyle D. Burgoon, PhD
Environmental Laboratory
US Army Engineer Research and Development Center
Research Triangle Park, NC

Sent from my BlackBerry 10 smartphone on the Verizon Wireless 4G LTE network.

From: Mark Harris
Sent: Friday, September 18, 2015 1:51 PM
To: Burgoon, Lyle D ERD-MS
Subject: [EXTERNAL] cr6study.info

Lyle: you should have received an approval for access to the data via email. If you have any issues downloading data just let me know. I did not realize you had changed jobs. Good luck with the new position. Why is the Army interested in Cr(VI)?

mark
--
ToxStrategies, Inc.

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Principal Health Scientist
~~~~~~

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Email: mharris@toxstrategies.com
Website: Blockedhttp://www.toxstrategies.com
Linkedin: Blockedhttp://www.linkedin.com/in/toxstrategiesharris
~~~~~~

Subject: Re: [EXTERNAL] cr6study.info
Date: Friday, January 8, 2016 at 5:04:08 PM Central Standard Time
From: Burgoon, Lyle D ERD-MS
To: Mark Harris

Thanks for the heads up, Mark.

Have a nice weekend.

Lyle D. Burgoon, PhD
Environmental Laboratory
US Army Engineer Research and Development Center
Research Triangle Park, NC

Sent from my BlackBerry 10 smartphone on the Verizon Wireless 4G LTE network.

From: Mark Harris
Sent: Friday, January 8, 2016 6:02 PM
To: Burgoon, Lyle D ERD-MS
Subject: Re: [EXTERNAL] cr6study.info

Lyle: we were doing some work on the Cr(VI) genomics data and noticed an error in the rat data (some probe ID's were mislabeled). The actual data is correct - just the labels are wrong. This doesn't impact our publications but does impact the data available for download from our Cr(VI) study website. MSU is fixing and I will alert you when we update the files.

mark

--

ToxStrategies, Inc.
Innovative Solutions > Sound Science

Mark Harris, Ph.D.
Principal Health Scientist

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Direct Dial: (281) 394-1567
Houston Office: (281) 712-2062 Ext 2001
Cell: (832) 868-7729
fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: Blockedhttp://www.toxstrategies.com
Linkedin: Blockedhttp://www.linkedin.com/in/toxstrategiesharris

From: "Burgoon, Lyle D ERD-MS" <Lyle.D.Burgoon@usace.army.mil>
Date: Friday, September 18, 2015 at 3:50 PM
To: Mark Harris <mharris@toxstrategies.com>
Subject: Re: [EXTERNAL] cr6study.info

Army moves paperwork pretty quick. If I know it's coming, I can grease the skids. If you have a tight turnaround on paperwork approval needed my lab director can expedite and maybe 1-2weeks we should be able to turn it around. Unless it's after July 31, then it might take longer bc of the backlog at contracts.

Lyle D. Burgoon, PhD
Environmental Laboratory
US Army Engineer Research and Development Center
Research Triangle Park, NC

Sent from my BlackBerry 10 smartphone on the Verizon Wireless 4G LTE network.

From: Mark Harris
Sent: Friday, September 18, 2015 4:40 PM
To: Burgoon, Lyle D ERD-MS
Subject: Re: [EXTERNAL] cr6study.info

Lyle: that is good to know about being able to collaborate on future projects. You never know when a client will ask for such analyses.

One question – is it is relatively straightforward to engage you all for a project? Meaning, if something came up, could the paperwork being done relatively quickly to get going?

mark

From: "Burgoon, Lyle D ERD-MS"
Date: Friday, September 18, 2015 at 3:35 PM
To: Mark Harris
Subject: Re: [EXTERNAL] cr6study.info

Hey Mark,

Thanks. The Army has no interest in chromium (thankfully). However, EPA is in the process of contracting with the Army to reanalyze the data. So, in preparation for that, we requested the data.

On a side note, in my new federal job I'm soft money, and we operate like a consulting company. So if in the future you need CompTox or toxicogenomic support, let me know. We can accept funds from companies for collaborative work.

If you want to chat sometime, my number is 517-303-5368.

Cheers,

Lyle

Lyle D. Burgoon, PhD
Environmental Laboratory

US Army Engineer Research and Development Center
Research Triangle Park, NC

Sent from my BlackBerry 10 smartphone on the Verizon Wireless 4G LTE network.

From: Mark Harris
Sent: Friday, September 18, 2015 1:51 PM
To: Burgoon, Lyle D ERD-MS
Subject: [EXTERNAL] cr6study.info

Lyle: you should have received an approval for access to the data via email. If you have any issues downloading data just let me know. I did not realize you had changed jobs. Good luck with the new position. Why is the Army interested in Cr(VI)?

mark
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Website: BlockedBlockedhttp://www.toxstrategies.com
LinkedIn: BlockedBlockedhttp://www.linkedin.com/in/toxstrategiesharris

Subject: Re: [EXTERNAL] cr6study.info
Date: Tuesday, April 5, 2016 at 2:42:13 PM Central Daylight Time
From: Burgoon, Lyle D ERD-MS
To: Mark Harris

Hey Mark,

Not started yet, but thanks for the update. Once I get cranking if I have questions I'll let ya know.

Cheers,

Lyle

Lyle D. Burgoon, PhD
Environmental Laboratory
US Army Engineer Research and Development Center
Research Triangle Park, NC

Sent from my BlackBerry 10 smartphone on the Verizon Wireless 4G LTE network.

From: Mark Harris
Sent: Tuesday, April 5, 2016 2:09 PM
To: Burgoon, Lyle D ERD-MS
Subject: Re: [EXTERNAL] cr6study.info

Lyle: just touching base. Have you done anything with the data – the labels are all fixed in the raw data.

Trust all is well.

mark

--

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notify ToxStrategies, Inc. at (832) 868-7729 and permanently delete the original and any copy of any e-mail and any printout thereof.

From: "Burgoon, Lyle D ERD-MS" <Lyle.D.Burgoon@usace.army.mil>
Date: Friday, January 8, 2016 at 5:04 PM
To: Mark Harris <mharris@toxstrategies.com>
Subject: Re: [EXTERNAL] cr6study.info

Thanks for the heads up, Mark.

Have a nice weekend.

Lyle D. Burgoon, PhD
Environmental Laboratory
US Army Engineer Research and Development Center
Research Triangle Park, NC

Sent from my BlackBerry 10 smartphone on the Verizon Wireless 4G LTE network.

From: Mark Harris
Sent: Friday, January 8, 2016 6:02 PM
To: Burgoon, Lyle D ERD-MS
Subject: Re: [EXTERNAL] cr6study.info

Lyle: we were doing some work on the Cr(VI) genomics data and noticed an error in the rat data (some probe ID's were mislabeled). The actual data is correct - just the labels are wrong. This doesn't impact our publications but does impact the data available for download from our Cr(VI) study website. MSU is fixing and I will alert you when we update the files.

mark

--
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LinkedIn: BlockedBlockedBlockedhttp://www.linkedin.com/in/toxstrategiesharris

Subject: Re: Cr(VI) raw microarray data
Date: Thursday, March 13, 2014 at 12:39:33 PM Central Daylight Time
From: Burgoon, Lyle
To: Mark Harris

Thanks, Mark; looking forward to the data. If you're going to SOT, safe travels.

Cheers,

Lyle

From: Mark Harris <mharris@toxstrategies.com>
Sent: Thursday, March 13, 2014 1:37:10 PM
To: Burgoon, Lyle
Cc: Thomas, Russell; Tim Zacharewski; Gibbons, Catherine; Chad Thompson; Deborah Proctor
Subject: Cr(VI) raw microarray data

Lyle: as I mentioned in the meeting on Monday, the data will be posted to our new Cr(VI) MOA website before SOT. Once the site is live I will send you all a link to it. You will be able to download the genomics data at that time.

The approach and methods that were used to analyze the data are adequately described in the Kopec papers. Our use of the genomics data to support the Mode of Action can be found in our Critical Reviews in Toxicology paper. Links to these Open Access papers can be found at the web page below:

http://www.toxstrategies.com/publications/CRVI_MOA_study.htm

mark

From: "Burgoon, Lyle" <Burgoon.Lyle@epa.gov>
Date: March 12, 2014 at 8:43:16 AM CDT
To: "cthompson@toxstrategies.com" <cthompson@toxstrategies.com>
Cc: "tzachare@msu.edu" <tzachare@msu.edu>, "Gibbons, Catherine" <Gibbons.Catherine@epa.gov>
Subject: Cr(VI) raw microarray data

Dr. Thompson,

It was nice seeing you and Dr. Harris (via video) at Monday's meeting @ NCEA HQ. I was quite pleased to hear Ms. Mason state that ACC expected the researchers would share their data and results with NCEA.

I'm following up on our discussion from Monday and am requesting access to all of your raw microarray data, as well as your analyzed data that supports the conclusions in your papers. In addition, it would be helpful if you could also supply us with the analysis code that was used,

any protocols used for the analyses, and any other supporting documentation that may help us understand how the assays and analyses were performed.

For clarity, I am using the MIAME definition of "raw data", and my request for the additional information is in line and keeping with the MIAME standard, which can be found here: http://www.mged.org/Workgroups/MIAME/miame.html.

To facilitate data transfer, I can set-up an EPA-based FTP site where you can upload the data.

Thanks again for presenting your latest results to us, and I look forward to receiving the data.

Cheers,

Lyle

Lyle D. Burgoon, Ph.D
National Center for Environmental Assessment
Chief, Hazardous Pollutant Assessment Group (Acting)
US Environmental Protection Agency
Phone: 919.541.7808
Fax: 919.685.3473
Cell: 919.397.8036

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Cell: (832) 868-7729
fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

Subject: Re: Cr(VI) raw microarray data
Date: Monday, March 17, 2014 at 9:40:37 AM Central Daylight Time
From: Burgoon, Lyle
To: mharris@toxstrategies.com
CC: tzachare@msu.edu, Gibbons, Catherine, cthompson@toxstrategies.com

Hi Mark,

I have read the Kopec, et al papers again, at your suggestion. Reading them reaffirms that we absolutely need to have the analysis code in order to understand what exactly was done. Per the MIAME standard (Minimum Information About a Microarray Experiment: http://www.mged.org/Workgroups/MIAME/miame_2.0.html):

> The essential laboratory and data processing protocols are usually described in the journal methods section. It is sufficient to simply reference the standard experimental or data processing protocols, such as MAS5 or RMA. However, if a protocol depends on parameters that can be varied, their values should be provided. If novel or non-standard data processing protocols are used, these should be described in sufficient detail to allow the user to understand what exactly has been done in the experiment and how the data have been analysed to reach the conclusions of the study.

In the case of the Kopec, et al papers, the analysis protocols used are non-standard data processing methods. As far as I can tell, these methods are commonly used in Dr. Zacharewski's laboratory; however, they are not commonly used elsewhere. The Eckel, et al 2005 and Eckel, et al 2004 papers describe analysis methods used in the Kopec, et al papers that require parameterization or changes to the code in order to work properly. Thus, it is critical for us to see the code in order to understand how the data were treated. Without the code we will be unable to understand how you came to draw the hypotheses you did in the Kopec, et al and subsequent papers. Without this understanding, we cannot interpret your results.

As MIAME is a community standard, first adopted several years ago by many prominent journals, you can see that this request is not unusual.

Likewise, we need to have the rawest form of the data possible for the QRT-PCR analyses, which Kopec, et al have used to support their microarray data. At a minimum, this would include any spreadsheets or text files of the data from the ABI machine, detailing all of the mathematical manipulations of the data from prior to standardization, through normalization, ratio calculation, and statistical analysis. Note: I couldn't find any details on how the statistical analysis of the QRT-PCR was performed, nor can I find a reference for how the standard errors of a ratio were calculated.

We may have additional data requirements and needs as we dig into the toxicogenomics data further, and we look forward to working with you in obtaining the information we need to better understand these studies.

Thanks,

Lyle

Lyle D. Burgoon, Ph.D
Chief, Hazardous Pollutant Assessment Group (Acting)
National Center for Environmental Assessment
US Environmental Protection Agency
Phone: 919.541.7808

Fax: 919.685.3473
Cell: 919.397.8036

From: Mark Harris <mharris@toxstrategies.com>
Sent: Thursday, March 13, 2014 1:37:10 PM
To: Burgoon, Lyle
Cc: Thomas, Russell; Tim Zacharewski; Gibbons, Catherine; Chad Thompson; Deborah Proctor
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Cell: (832) 868-7729
fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

Subject: RE: Cr(VI) MOA Study Website
Date: Thursday, April 3, 2014 at 2:59:52 PM Central Daylight Time
From: Burgoon, Lyle
To: Mark Harris
CC: Gibbons, Catherine, Vandenberg, John, Flowers, Lynn

Hi Mark,

We cannot access the data at this time due to our inability to agree to the Terms and Conditions associated with data access found here: http://cr6study.info/study-data/terms-and-conditions/

Specifically, we request that ToxStrategies delete the indemnification clause in the Terms and Conditions. As written, the Agency cannot agree to the terms as it would be a violation of the Anti-Deficiency Act (i.e., making a commitment to an unknown future cost is a direct violation of the Anti-Deficiency Act).

Please let me know what action you are willing to take as soon as possible. As you are aware, time is of the essence with respect to the Cr(VI) IRIS Assessment. Your prompt attention to this matter is appreciated.

Thanks,

Lyle

Lyle D. Burgoon, Ph.D
Chief, Hazardous Pollutant Assessment Group (Acting)
National Center for Environmental Assessment
US Environmental Protection Agency
Phone: 919.541.7808
Fax: 919.685.3473
Cell: 919.397.8036

From: Mark Harris [mailto:mharris@toxstrategies.com]
Sent: Monday, March 24, 2014 9:34 AM
To: Burgoon, Lyle
Cc: Tim Zacharewski; Gibbons, Catherine; Chad Thompson; Thomas, Russell
Subject: Cr(VI) MOA Study Website

Lyle: our Cr(VI) MOA study website is live. You can access is at www.cr6study.info

Under the Study Data tab you can register to download data. Once registered, you will receive a password to access the data download portion of the site.

Not all of the genomics data you requested has been posted. Things that are not posted yet are:

1. Analysis Code
2. QRT-PCR data

These items will be added in the next few weeks.

mark

--

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Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

Subject: RE: T&C - how does this
Date: Monday, April 28, 2014 at 10:08:36 AM Central Daylight Time
From: Burgoon, Lyle
To: Mark Harris

Hi Mark,

Thanks for working with us – that language meets our needs. Sorry for the delay – was out sick last week.

Cheers,

Lyle

Lyle D. Burgoon, Ph.D
Chief, Hazardous Pollutant Assessment Group (Acting)
National Center for Environmental Assessment
US Environmental Protection Agency
Phone: 919.541.7808
Fax: 919.685.3473
Cell: 919.397.8036

From: Mark Harris [mailto:mharris@toxstrategies.com]
Sent: Wednesday, April 23, 2014 3:09 PM
To: Burgoon, Lyle
Subject: T&C - how does this

Lyle: I got some revised T&C language from the attorneys today. See attached in Tracked Changes. If this works let me know and I will get the website updated.

Also, if these words don't work, can you ask the attorney reviewing this to suggest some words that would work for the US government?

thanks

mark

--

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Subject: Re: [EXTERNAL] cr6study.info
Date: Friday, September 18, 2015 at 3:35:41 PM Central Daylight Time
From: Burgoon, Lyle D ERD-MS
To: Mark Harris

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Subject: Re: [EXTERNAL] cr6study.info
Date: Friday, September 18, 2015 at 3:50:25 PM Central Daylight Time
From: Burgoon, Lyle D ERD-MS
To: Mark Harris

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Email: mharris@toxstrategies.com
Website: Blockedhttp://www.toxstrategies.com
Linkedin: Blockedhttp://www.linkedin.com/in/toxstrategiesharris

Subject: New user registration for Cr(VI) MOA Study
Date: Wednesday, August 10, 2016 at 1:16:17 PM Central Daylight Time
From: CrVI MOA Study
To: mharris@toxstrategies.com

The following user registered for Cr(VI) MOA Study (and is pending approval)

username: sfoster
email: foster.stiven@epa.gov

First Name: Stiven
Last Name: Foster
Company: USEPA
Position: Toxicologist
Address 1: 1200 Pennsylvania Ave, NW
Address 2: MC-5103T
City: Washington
State: DC
Zip: 20460
Country: USA
Day Phone: 202-566-1911
Plans for Data Use: Evaluation of finding for consideration in EPA efforts.
TOS: agree

This user registered here:
http://cr6study.info/register/

user IP: 161.80.120.176

activate user: http://cr6study.info/wp-admin/user-edit.php?user_id=69

This is an automated message from Cr(VI) MOA Study
Please do not reply to this address

Subject: (none)
Date: Wednesday, August 10, 2016 at 1:19:59 PM Central Daylight Time
From: Mark Harris
To: foster.stiven@epa.gov

I have activated your access to the raw data. If you have any problems accessing the data let me know.

Mark

--
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